AR201-14112B

Substance Group:	**Group 16**
Summary Prepared by:	**Petroleum Additives Panel** **Health & Environmental Research Task Group**
Date of last update:	**November 22, 2002**
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1.0 Biodegradation

Test Substance	
CAS #	27859-58-1
Chemical Name	Butanedioic acid, (tetrapropenyl)-
Remarks	Test material purity not provided
Method	
Method/Guideline followed	OECD 301F
Test Type (aerobic/anaerobic)	Aerobic Manometric Respirometry Test (Biodegradation)
GLP (Y/N)	Y
Year (Study Performed)	1999
Contact time (units)	28 days.
Inoculum	Activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Test System: The test system was a defined mineral medium inoculated with the supernatant of homogenized activated return sludge from a public wastewater treatment plant. The mineral medium was prepared as outlined in OECD Guideline 301F. Inoculum: The supernatant from homogenized activated sludge was used as inoculum. A two-liter flask containing 100 mL of supplemented sludge supernatant and 900 mL of test medium was prepared. The inoculum was pre-adapted to the test material for 14 days during which the test substance was added incrementally at concentrations equivalent to 4, 8, and 8 mg carbon/L on days 0, 7, and 11, respectively. The targeted microbial level in the test mixture was 10,000 to 1,000,000 cells/mL. The actual microbial level in the test mixture was 1000 cells/mL. This deviation from the protocol was not considered significant. Concentration of test chemical (assay conducted in duplicate reactor flasks): Test substance concentrations were 107.2 and 110.2 mg/L, giving a 122.1 and 125.5 mg ThOD. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a Teflon coupon and introduced into the medium. Test mixtures were stirred throughout the study using magnetic stirrers. Temp of incubation: 23 ± 1ºC Dosing procedure: A measured volume of the inoculated mineral medium containing approximately 107-110 mg/L test substance was continuously stirred in a closed system for 28 days. Sampling frequency: The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test. Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control. Analytical method: Oxygen uptake was measured using a BI-1000 electrolytic respirometer system. The hydrogen, nitrogen and total organic carbon content of the test material were determined.

	Method of calculating measured concentrations: Test material concentrations were not measured.
Results	Test Validity: All test validity criteria were met as follows: The average oxygen uptake of each of the two inoculum blanks was lower than 60 mg/L in 28 days. The difference in biodegradation levels of the reference and test substance replicates was less than 20%. The percent degradation of the reference material reached the pass level (60%) within 14 days. The final pH of the test mixtures were within the range of 6.0-8.5 demonstrating the biodegradation was not inhibited by extreme pH.
Degradation % after time	Test substance: 18.3% after 28 days Positive reference (sodium benzoate): – >60% (3d)
Remarks	
Conclusions	18.3% after 28 days. The reference substance, sodium benzoate, reached a level of 94.2% in the same test period.
Data Quality	(1) Reliable without restriction
References	Unpublished Confidential Business Information.
Other	Updated: 11/5/2002

AQUATIC ORGANISMS

2.3 Acute Toxicity to Aquatic Plants (e.g. algae)

Robust Summary #: 16-ALG-1

Test Substance	
CAS #	27859-58-1
Chemical Name	Butanedioic acid, (tetrapropenyl)-
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1993), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	***Freshwater algae,* Pseudokirchneriella subcapitata *formerly called* Selenastrum capricornutum**
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	No
Statistical methods	Average specific growth rate was calculated as the natural log of the number of cells/ml at 72 and 96 hours minus the natural log of the number of cells/ml at 0 hour, divided by the hour of exposure. Results were interpreted by standard statistical techniques. All calculations were performed using nominal concentrations of the test material with the number of cells/mL, then with the average specific growth rates.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from the University of Texas at Austin alga collection. Test System: Each WAF was prepared only at the beginning of the test. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 20 hours. Stirring accomplished using a magnetic stirrer. Mixing speed was adjusted such that a vortex formed approximately 25% of the distance to the bottom. Following the mixing period, the test solution was allowed to stand for 4 hour before the water phase was removed. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were covered to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). The occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers or

	aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Cell counts were made at 72 and 96 hours. Light: Cool-white fluorescent lights provided a light intensity of approximately 400-430 foot-candles. Test temperature: 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978) adjusted to pH 7.5. Measured total suspended solids in fresh untreated alga media were <10 mg/L, respectively. Test media pH was 7.4 at 0-hour and 10.2 after 96 hours. Test Levels: Control and 0.3, 3.0, 33, 330 and 3300 mg/L WAF loading rates. Insoluble material was observed at 24, 48 and 96 hours in test vessels containing 330 and 3300 mg/L. No other insoluble material was observed during the study. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: Not performed concentrations were all based on nominal.
Results	96-h EC_{50} 93 mg/L; The 96-hr NOEC = 33 mg/L.
Remarks	Test Findings: Insoluble material was observed at 24, 48 and 96 hours in test vessels containing 330 and 3300 mg/L. No other insoluble material was observed during the study. The algal population grew well resulting in an average of 1,508,000 cells/mL in the control after 96 hours. Water quality was acceptable throughout the study. The two highest concentrations of test material significantly decreased the pH of the test media at the beginning of the test (330 mg/L pH: 4.3-4.4; 3000 mg/L pH: 3.9-4.0. No biological effects were noted during the study on cell size, shape, color, flocculation, adherence to test containers or aggregation. The 96-hour no observed effect concentration (NOEC) was 33 mg/L. The calculated EC50s were as follows: Based on Number of Cells/mL 72 hr EC50: 100 mg/L (95% confidence interval 33-330 mg/L) 96 hr EC50: 93 mg/L (95% confidence interval 33-330 mg/L) Based on Average Specific Growth Rate 72 hr EC50: 100 mg/L (95% confidence interval 33-330 mg/L) 96 hr EC50: 100 mg/L (95% confidence interval 33-330 mg/L) • The toxic effects were determined to be algistatic based on the rapid re-growth of an aliquot of cells taken from the 330mg/L test vessel and cultured in fresh control media. • Control response was satisfactory.

Conclusions	The test material was considered algistatic to freshwater alga. at loading rates of 330 and 3000 mg/L. 96-h EC_{50} 93 mg/L; The 96-hr NOEC = 33 mg/L.
Data Quality	(1) Reliable with restriction. Restriction due to the lack of any analytical confirmation of test material concentration in test solutions. All concentrations are expressed as nominal.
References	Confidential business information.
Other	Updated: 11/4/2002

3.1 Acute Toxicity

3.1.1 Acute Oral Toxicity

Test Substance	
CAS #	CAS# 25377-73-5
Chemical Name	Succinic anhydride, dodecenyl-
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/Sherman-Wistar
Sex	Male
No. of animals/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1, 2, 4, 8 and 16 g/kg
Dose volume	Not Provided
Vehicle control group	None
Chemical analysis of dosing solution	No
Remarks field for test conditions	(Note: This study was conducted several years prior to the establishment of this test guideline. This report provides a summary of study findings. Individual data are not presented.) A single administration of the test material was given intragastrically to five fasted male rats at each dose level. The animals were observed for signs of toxicity or behavioral changes on the day of treatment and throughout the 14-day observation period. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals.
Results	LD50 = 2.9 (2-4) g/kg (males)
Remarks	During the first three days of study all animals treated at the 4, 8 and 16 g/kg dose levels died. No deaths were observed at the 1 and 2 g/kg dose levels. No clinical signs of toxicity were observed at 1 g/kg. At 2 g/kg the animals were lethargic and had an oily appearance for up to 48 hours post dosing. All animals at the 4, 8 and 16 g/kg dose levels were severely depressed prior to death. No body weight effects occurred at 1 or 2 g/kg. Body weight data was not available at higher dose levels due to the observed mortality. No test material related macroscopic findings were evident.

Conclusions	The test article, when administered to 5 male rats/ dose group, had an acute oral LD50 of 2.9 g/kg.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report..
References	Unpublished confidential business information
Other	Updated: 2/23/01 (RTA-074)